PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12M 1/02	A1	(11) International Publication Number: WO 96/41864
		(43) International Publication Date: 27 December 1996 (27.12.96)

(21) International Application Number: PCT/IB96/00631

(22) International Filing Date: 10 June 1996 (10.06.96)

(30) Priority Data:
08/489,819 13 June 1995 (13.06.95) US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US).

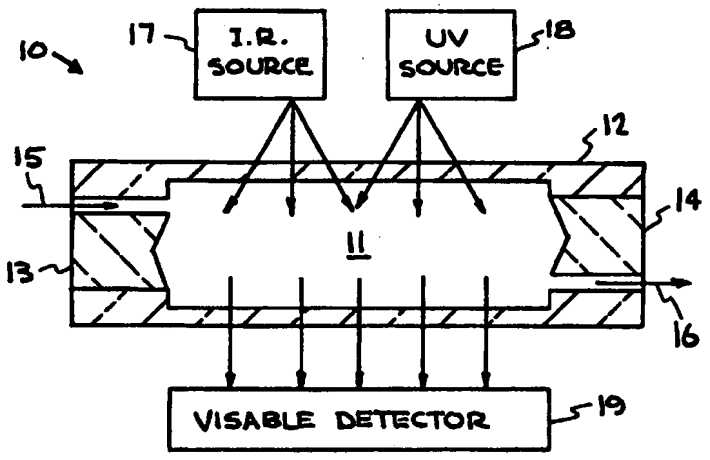
(72) Inventors: NORTHRUP, Milton, A.; 923 Creston Road, Berkeley, CA 94908 (US). MARIELLA, Raymond, P., Jr.; 872 Quanito Court, Danville, CA 94526 (US). KOO, Jackson, C.; 601 Van Ness Avenue #349, San Francisco, CA 94102 (US). DAVIDSON, J., Courtney; 449 Humboldt Way, Livermore, CA 94550 (US).

(74) Agent: SARTORIO, Henry, P.; P.O. Box 808, L-703, Livermore, CA 94550 (US).

(81) Designated States: CA, JP, KR, SG, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DIODE LASER HEATED MICRO-REACTION CHAMBER WITH SAMPLE DETECTION MEANS



(57) Abstract

A heat source (17, 18) and detector (19) arrangement for a miniaturized, microfabricated instrument (10) in which a polymerase chain reaction (PCR) is carried out. PCR is a bioanalytical method that uses enzymes and other reagents to amplify DNA or RNA from undetectable amounts to very detectable amounts, through a thermal cycling technique. Small IR or UV sources (17, 18) are used as heat sources for the PCR thermal cycle of the PCR technique, and sample conformation within the reaction chamber is established by detectors (19). Detection can be established when emitted light is absorbed by the DNA molecule, for example, or when a UV source induces fluorescence of the PCR products in the visible spectrum.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

DIODE LASER HEATED MICRO-REACTION CHAMBER WITH SAMPLE DETECTION MEANS

The United States Government has rights in this invention pursuant to Contract No. W-7405-ENG-48 between the United States Department of Energy and the University of California for the operation of Lawrence Livermore National Laboratory.

BACKGROUND OF THE INVENTION

The present invention relates to instruments for chemical reaction control and detection of participating reactants and resultant products, particularly to integrated microfabricated instruments for performing microscale chemical reactions involving precise control of parameters of the reactions, and more particularly to a Laser-diode based heated micro-reaction chamber and integrated means of nucleic acid detection therein.

Microfabrication technologies are now well known and include sputtering, electrodeposition, low-pressure vapor deposition, photolithography, and etching. Microfabricated devices are usually formed on crystalline substrates, such as silicon and gallium arsenide, but may be formed on non-crystalline materials, such as glass or certain polymers. The shapes of crystalline devices can be precisely controlled since etched surfaces are generally crystal planes, and crystalline materials may be bonded by processes such as fusion at elevated temperatures, anodic bonding, or field-assisted methods.

Monolithic microfabrication technology now enables the production of electrical, mechanical, electromechanical, optical, chemical and thermal devices, including pumps, valves, heaters, mixers, and detectors for microliter to nanoliter quantities of gases, liquids, and solids. Also, optical waveguide probes and ultrasonic flexural-wave sensors can now be produced on a microscale. The integration of these microfabricated devices into a single system allows for the batch production of microscale reactor-based analytical instruments. Such

integrated microinstruments may be applied to biochemical, inorganic, or organic chemical reactions to perform biomedical and environmental diagnostics, as well as biotechnological processing and detection.

The operation of such integrated microinstruments is easily automated, and since the analysis can be performed in situ, contamination is very low. Because of the inherently small sizes of such devices, the heating and cooling can be extremely rapid. These smaller devices have reduced power requirements and can be powered by batteries or by electromagnetic, capacitive, inductive or optical coupling.

The small volumes and high surface-area to volume ratios of microfabricated reaction instruments provide a high level of control of the parameters of a reaction. Heaters may produce temperature cycling or ramping; while sonochemical and sonophysical changes in conformational structures may be produced by ultrasound transducers; and polymerizations may be generated by incident optical radiation.

Synthesis reactions, and especially synthesis chain reactions such as the polymerase chain reaction (PCR), are particularly well-suited for microfabrication reaction instruments. PCR can selectively amplify a single molecule of DNA (or RNA) of an organism by a factor of 10^6 to 10^9. This well-established procedure requires the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of an original DNA target molecule, specific DNA primers, deoxynucleotide triphosphates, and DNA polymerase enzymes and cofactors. Each cycle produces a doubling of the target DNA sequence, leading to an exponential accumulation of the target sequence.

The PCR procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract; 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and oligonucleotide primers; 3) thermal cycling of the reaction mixture between two or three temperatures (e.g., 90-96, 72, and 37-55°C); and 4) detection of amplified DNA or RNA. Intermediate steps, such as purification of the reaction products and the incorporation of surface-bending primers, for example, may be incorporated in the PCR procedure.

A problem with standard PCR laboratory techniques is that the PCR reactions may be contaminated or inhibited by the introduction of a single contaminant molecule of extraneous DNA, such as those

from previous experiments, or other contaminants, during transfers of reagents from one vessel to another. Also, PCR reaction volumes used in standard laboratory techniques are typically on the order of 50 microliters. A thermal cycle typically consists of four stages: heating a sample to a first temperature for denaturation of the DNA, maintaining the sample at the first temperature, cooling the sample to a second lower temperature for extension or annealing of the DNA, and maintaining the temperature at that lower temperature. Typically, each of these four stages of a thermal cycle requires about one minute, and thus to complete forty cycles, for example, requires about three hours. Thus, due to the large volume typically used in standard laboratory procedures, the time involved, as well as the contamination possibilities during transfers of reagents from one vessel to another, there is clearly a need for microinstruments capable of carrying out the PCR procedure, particularly if the reaction vessel may be disposable. Recently, the cycling time for performing the PCR reaction has been reduced by performing the PCR reaction in capillary tubes and using a forced air heater to heat the tubes. Also, an integrated microfabricated reactor has been recently developed for in situ chemical reactions, which is especially advantageous for biochemical reactions which require high-precision thermal cycling, particularly DNA-based manipulations such as PCR, since the small dimensions of microinstrumentation promote rapid cycling times. The microfabricated reactor is described and claimed in copending U.S. Application Serial No. 07/938,106, filed August 31, 1992, entitled "Microfabricated Reactor", assigned to the same assignee.

The present invention involves an improvement which can be utilized in the reactor of the above-identified copending application, but also can be utilized in other miniaturized, microfabricated instruments. This invention utilizes an optically-heated and optically interrogated micro-reaction chamber, such as used in the PCR process. The present invention is directed to heating and nucleic acid detection in a miniaturized, microfabricated instrument. The invention utilizes a miniature heat source, such as an infrared (IR) source or an ultra-violet (UV) source, to heat a reaction chamber. Also, light emitting diodes and detectors are used to confirm the presence of a desired sample, such as a DNA molecule or selected dye. There are many uses of such optically-heated and optically interrogated micro-

reaction chambers. Also, optical energy can be used directly to induce or catalyze the reaction (UV/IR cross-linking, polymerizations). In addition to IR and UV sources, a visible (Vis) source can be utilized for detection and/or monitoring the reaction.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide heating means for an integrated microfabricated reactor.

A further object of the invention is to provide detection means to confirm the presence of a desired sample in a reactor-based instrument for inorganic, organic, and biochemical reaction.

A further object of the invention is to provide an optically-heated and optically interrogated micro-reaction chamber in which ultra violet, visible and infrared spectrums can be used in combination for heating, inducing reactions directly, or for detecting reaction progress or products.

Another object of the invention is to provide a microfabricated reactor with rapid high-precision thermal cycling, using a laser-diode based heated reaction chamber.

Another object of the invention is to provide an IR or UV source as a heater in a thermal cycle, such as used in the polymerase chain reaction (PCR).

Another object of the invention is to provide a UV heating source and a detector for the reaction products in the visible spectrum.

Another object of the invention is to provide IR, Vis or UV light emitting diodes and detectors in an IR or UV heated chemical reaction chamber to confirm the presence of a desired sample.

Another object of the invention is to provide a chemical reaction chamber in which the ultra-violet, visible, and infrared spectrums can be utilized for detection and/or monitoring the reaction in the chamber.

Other objects and advantages of the invention will become apparent from the following description and accompanying drawings. The invention basically involves a bulk-heater source which will circumvent the limitations of surface heating which relies on thermal conduction of the liquid. The invention utilizes a diode laser that is designed to emit energy at a selected wavelength to provide a miniature heat source for thermal cycling in a chemical reaction chamber, such as

used in the PCR process. The invention additionally involves a detector arrangement to confirm the presence of desired reaction products. The present invention may be utilized, for example, in the integrated microfabricated reactor of above-referenced copending application S.N. 07/938,106 to replace the reaction chamber heating arrangement thereof and to incorporate sample detection within the reaction chamber, or the invention can be utilized with other miniaturized reaction chambers such as in the case of synthetic reactions that require heat, light, or thermal cycling. Thus, the invention can be utilized in microinstruments being developed for genetic analysis for the Human Genome Project or for forensic analysis based on DNA detection, or other miniaturized instruments for DNA/RNA or other biomolecule analysis and detection.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the disclosure, illustrate embodiments of the invention and, together with the description, serve to explain the principles of the invention.

Figure 1 schematically illustrates an embodiment of a heating and detection arrangement for a microfabricated reaction chamber, such as a PCR chamber, made in accordance with the present invention.

Figure 2 schematically illustrates another embodiment of a reaction chamber of an instrument, such as a PCR microinstrument with heating and detection means in accordance with the present invention.

Figure 3 is a graph showing laser absorption and tissue penetration using wavelength vs. absorption coefficient of the UV-IR spectrum.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to optically-heated and optically integrated microfabricated instruments that perform reactant and product manipulations and detection on microliter to picoliter samples. By the selection and integration of appropriate microfabricated device, a precise and reliable reaction and analysis instrument for reaction-based diagnostics, such as PCR, is implemented. The present invention involves heating and detection arrangements for a chemical reaction chamber.

There are many uses of such an optically-heated and optically interrogated micro-reaction chambers, which include: organic synthesis reactions that use either or both thermally and light-induced reactions and detections, bioreactors for biosynthesis (enzymes, etc.), photolysis, and photosynthesis. Optical energy can be used to directly induce or catalyze the reaction (UV/IR cross-linking, polymerizations). It can be used as a micro-incubator for growth of microorganisms for bacterial assays in the field.

The light can be used to heat the reagents to a thermally-induced reaction, for example. This would occur in several ways: 1) the light heats up the water-based media directly, which works efficiently at wavelengths from 1.5-4.0 microns (1500 to 4000 nm) because at these wavelengths silicon is a transparent window; and 2) the reaction could contain optical absorbers which convert the optical energy to heat, that will therefore heat the reaction mixture. There are efficient absorbers and (converters to heat, such as carbon particles) in the UV, visible, and IR spectrums which also are all potentially useful for detection and/or monitoring the reaction. Also, thinning would cause less energy to go into the walls of the reactor by conduction plus silicon's heat capacity.

The terms PCR chamber, PCR reaction, or PCR products, are used herein only as examples of the reaction chamber and reactions, reagents, and products of chemical reactions, due to the PCR process being well known. A PCR chamber is basically, in the case of synthetic reactions, a chamber that requires heat, light, or thermal cycling. The PCR process basically involves control, augmentation, and monitoring of chemical reactions.

The heater arrangement will be first described, followed by a description of the detection arrangement.

The polymerase chain reaction (PCR), for example, is a bioanalytical method that uses enzymes and other reagents to amplify DNA or RNA, for example, from undetectable amounts to very detectable amounts, through a thermal cycling technique. The thermal cycling of the reaction mixture is between two or three temperatures, and thus the effectiveness of the heater arrangement is of substantial importance, whereby the thermal cycling time can constitute a relative short time period.

The present invention utilizes an IR source as the heating source for the reaction chamber and a UV source for detection. By way of example, the UV source could be a lamp, the Vis source could be a lamp or diode-laser, and the IR source could be a laser diode. Diode-laser, solution absorbent-based heating of a liquid will provide rapid and uniform heat distribution for material in a thermally driven chemical/physical reaction chamber. Existing PCR systems heat the vessel walls, not the reaction mixture directly. Water, for example, absorbs radiant energy effectively at various wavelengths (1.5 μm to 4.0 μm, for example). A semiconductor/diode-laser which is made to emit energy at that wavelength provides a miniature heat source. Such a bulk-heater source would circumvent the limitations of surface heating, which relies on thermal conduction of the liquid. The heater of this invention is a IR diode-laser designed to emit energy at a selected wavelength so as to effectively heat the specific solution in a PCR or other chemical reaction chamber.

Referring now to Figure 1, a chemical reaction chamber, such as a PCR chamber, of a miniaturized, microfabricated instrument, generally indicated at 10 is illustrated in cross-section, with the chamber 11 being formed in a housing 12, constructed of Pyrex for example, and having a silicon walls 13 and 14 therein, with an inlet 15 and an outlet 16. Two different laser-diode heating sources are illustrated, one source 17 being an infrared (IR) source, and the second source 18 being an ultra-violet (UV) source. The IR heating source 17 applies heat more uniformly through the bulk of the solution in chamber 11. The UV heating source 18 induces fluorescence of the reaction products in the visible (Vis) spectrum, which can be detected by a visible (Vis) detector 19 located external of the housing 12 defining reaction chamber 11. It is understood that housing 12 must be constructed of a material transparent to UV and/or visible spectrum.

Figure 2 schematically illustrates an instrument, such as a PCR microinstrument 20, having a reaction chamber 21 heated by an IR heating source 22 and provided with a diode light source 23 and a solid state detector 24. The light source 23 and detector 24 may each be provided with an appropriate filter.

Detection of the amplified product in a PCR chamber typically requires removing the sample and running it on an

electrophoresis gel. Recently, researchers have developed a real-time, in situ, method that monitors the about 620 nm emission of Ethidium Bromide DNA intercalating dye, during the PCR cycling. (Higuchi et al, Bio/technology, Vol. 11, Sept. 1993, pg. 1026). By this invention, and the use of an optical heating source, detection of the reaction products can be obtained in the visible spectrum, as described above with respect to Figure 1. Also, by incorporating an integrated excitation and detection system in the reaction chamber itself, confirmation of the presence of a sample can be obtained (Scientific American, January 1994, pp. 149-150). The detection arrangement illustrated in Figure 2 utilizes specific light-emitting diodes (LEDs) and diode detectors, with or without filters, which allow significant miniaturization and integration, and therefore an increased efficiency, to allow development of a complete integrated miniature instrument, as illustrated in Figure 2. Referring again to Figure 2, the diode light source 23, such as an LED, emits light which is absorbed by the DNA molecule or an intercalating or chemically-linked dye. One example is the intercalating dye Ethidium Bromide, which absorbs in both the UV (around 300-400 nm) and the visible (500-590 nm). In one embodiment a green diode (around 540 nm) could be used as a light source. The detection of the DNA/dye complex is accomplished by detector 24 via detection of the emission of light from the fluorescent molecular tag. For example, Ethidium Bromide emits light around 620 nm, which could be detected with a diode detector.

One specific example is as follows: The PCR reaction is in buffered salt water, a wavelength of 1.5 μm is and 380 nm (or 540 nm) to fluorescence a Ethidium Bromide dye which intercalates into the DNA as it is being made and monitor it at 630 nm. In this way two wavelengths are being used, one to heat and one to induce fluorescence. Similar approaches, but different wavelengths can be applied to many other reactions.

The diode-light source 23 and detector 24 may be provided is appropriate interference optical or colored glass filters. For example, the filter for light source 23 may be 488 nm ± 50 nm; while the filter for detector 24 may be 620 nm ± 20 nm.

By way of example, the IR heating source 17 operating in the 0.7-10.0 μm (700-10,000 nm) wavelength range has an emitted power range of 0.1 to 10 watts which can be utilized with materials (reagents,

solutions, etc) such as aqueous solutions of reactants. Also, by the use of certain dyes, the optical heating source can also be used as a light emitter which can be detected via fluorescent molecular tagging. By way of example, the UV heating source 18 has a wavelength in the range of 0.18-0.4 μm (180 nm to 400 nm), with an energy range of 0.1 to 10 watts which can be utilized to heat silicon and/or certain converters, but not water. The visible (Vis) source operates in the 0.4-0.7 μm (400-700 nm) range and has an energy when used with converters in the range of 0.1 to 10 watts. Water does not absorb in near UV and visible ranges.

The following table sets forth detection/heat input and detection output for each of the UV, Visible and IR sources:

	UV (100-400 nm)	Visible (400-600 nm)	IR (0.6-4.0 μm)
Detection/Heat Input	.Heating of absorbants/converters .Excitation of fluorescent dyes[1]	.Heating of absorbants/converters[2] .Excitation of fluorescent dyes[4] .Absorption by reagents	.Heating of water directly[5] or absorbants/converters .Excitation of fluorescent dyes
Detection Output	.Absorption by reagents[3]	.Emission of dyes	.Emission of dyes[1]

1. ETBr, or other DNA intercalating dyes
2. Malachite green, carbon black, silicon, other suspended absorber materials
3. Biological material absorption at 260-290 nm
4. ETBr, covalently-bound DNA dyes
5. 1.5 μm wavelength

Figure 3 shows the laser absorption and tissue penetration using an Ho:YAG laser by wavelength vs. absorption coefficient, and which illustrates the water heating at 1.5 μm and above.

By the use of diode-laser heating sources and excitation and detection arrangements for the reaction chamber a micro-reaction chamber would allow the real-time, integrated detection of DNA when it is being produced by a chemical reaction such as the PCR technique within the microfabricated chamber. Such a combination of heating and

detection arrangements will advance the state of miniaturized, microfabricated instruments.

It has thus been shown that by the use of laser-diode heating source alone or in combination with light detecting arrangements, the present invention enables rapid and uniform heating for the thermal cycle of a chemical reaction, such as the PCR procedure. The heating sources, under certain circumstances additionally function as a light emitting source for excitation and detection via light absorbed by the DNA/dye. By the integration of the heating/detection arrangements of this invention into a PCR microinstrument, for example, uniform rapid heating and real-time detection can be accomplished.

While particular embodiments, materials, parameters, etc. have been illustrated and/or described to set forth examples and a full understanding of the invention, such are not intended to be limiting. Modifications and changes may become apparent to those skilled to the art, and the invention is to be limited only by the scope of the appended claims.

CLAIMS

1. In a microfabricated instrument having a chamber for control, augmentation, and monitoring of chemical reactions, the improvement comprising:

a heating source for heating said chamber selected from the group consisting of an infrared source and an ultra-violet source.

2. The improved instrument of Claim 1, additionally including means for confirming the presence of a sample in said chamber.

3. The improved instrument of Claim 2, wherein said means comprises a light emitting means and a detector means, using a light spectrum selected from the group of ultra-violet, visible, and infrared ranges.

4. The improved instrument of Claim 3, wherein said heating source comprises said light emitting means for said means for confirming the presence of a sample in said chamber.

5. The improved instrument of Claim 4, wherein said heating source is an ultra-violet source and said detector means is a visible detector.

6. The improved instrument of Claim 3, wherein said light emitting means comprises a light-emitting diode, and wherein said detector means comprises a solid state diode detector.

7. The improved instrument of Claim 1, wherein said heating source is an infrared source emitting power in the range of 0.1 to 10 watts, with a wavelength of 0.7 to 10.0 μm.

8. The improved instrument of Claim 1, wherein said heating source is an ultra-violet source with a wavelength of 180 nm to 400 nm.

9. The improvement of Claim 1, wherein said heating source is a visible source with a wavelength of 400 nm to 700 nm.

10. In a microfabricated reactor using thermal cycling of a reaction mixture between two to three temperatures, the improvement comprising a diode-laser heating source.

11. The improvement of Claim 10, wherein said diode-laser heating source is selected from the group consisting of an infrared source and an ultra-violet source.

12. The improvement of Claim 10, additionally including means for excitation and means for detection of a material in the reaction mixture.

13. The improvement of Claim 12, wherein said heating source comprises said means for excitation.

14. The improvement of Claim 12, wherein said means for excitation comprises a light-emitting diode.

15. The improvement of Claim 14, wherein said means for detection comprises a diode detector.

16. The improvement of Claim 13, wherein said means for detection comprises a visible spectrum detector.

17. The improvement of Claim 10, wherein the diode-laser is composed of a UV, VIS, and IR source cable of use in combination for heating, inducing reactions directly, or for deleting reaction progress or products.

18. A diode-laser based heated micro-reaction chamber, using direct or indirected optical heating, wherein said diode-laser is selected from the group consisting of an infrared, visible, and ultra-violet heating source.

19. The micro-reaction chamber of Claim 17, additionally including an integrated excitation and detection system in the micro-reaction chamber.

20. The micro-reaction chamber of Claim 18, wherein said integrated excitation and detection system is composed of an ultra-violet heating source and a visible spectrum detector.

21. The diode-laser based heated micro-reaction chamber of Claim 17, wherein the indirect optical heating utilizes absorbents/converters selected from malachile green dye and carbon particles.

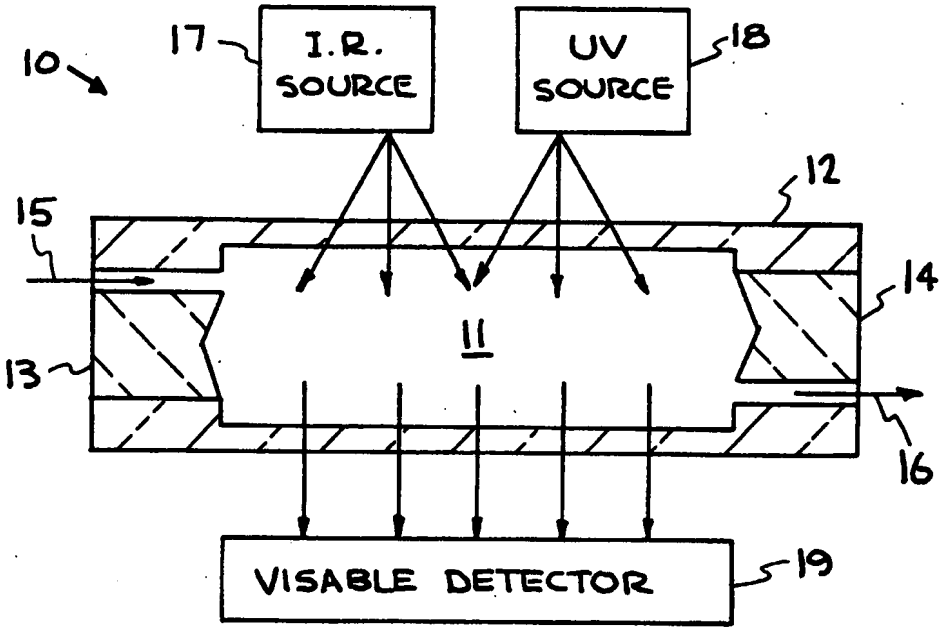

10
17
I.R. SOURCE
UV SOURCE
18
12
15
14
11
13
16
VISABLE DETECTOR
19

FIG. 1

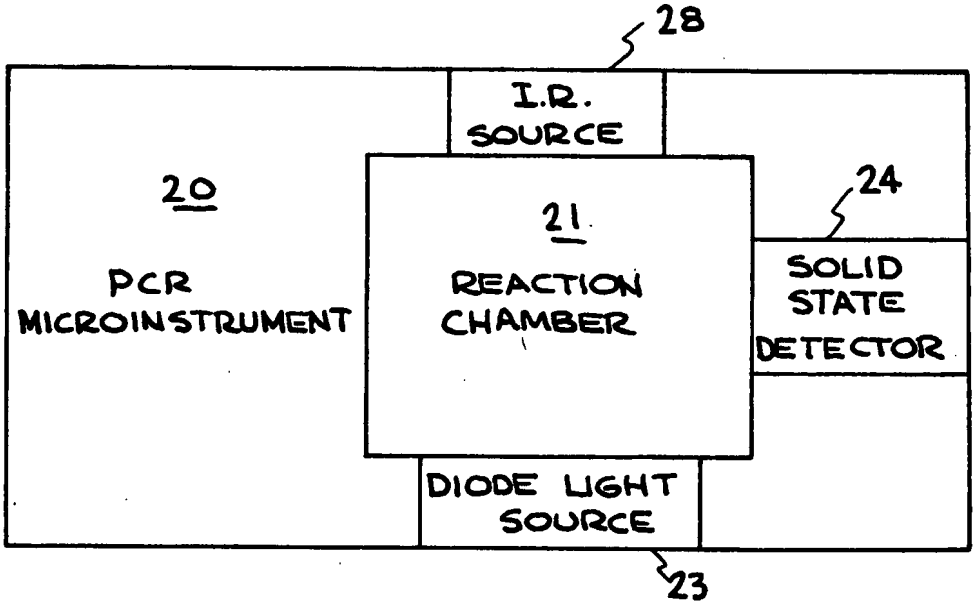

28
I.R. SOURCE
20
PCR MICROINSTRUMENT
21
REACTION CHAMBER
24
SOLID STATE DETECTOR
DIODE LIGHT SOURCE
23

FIG. 2

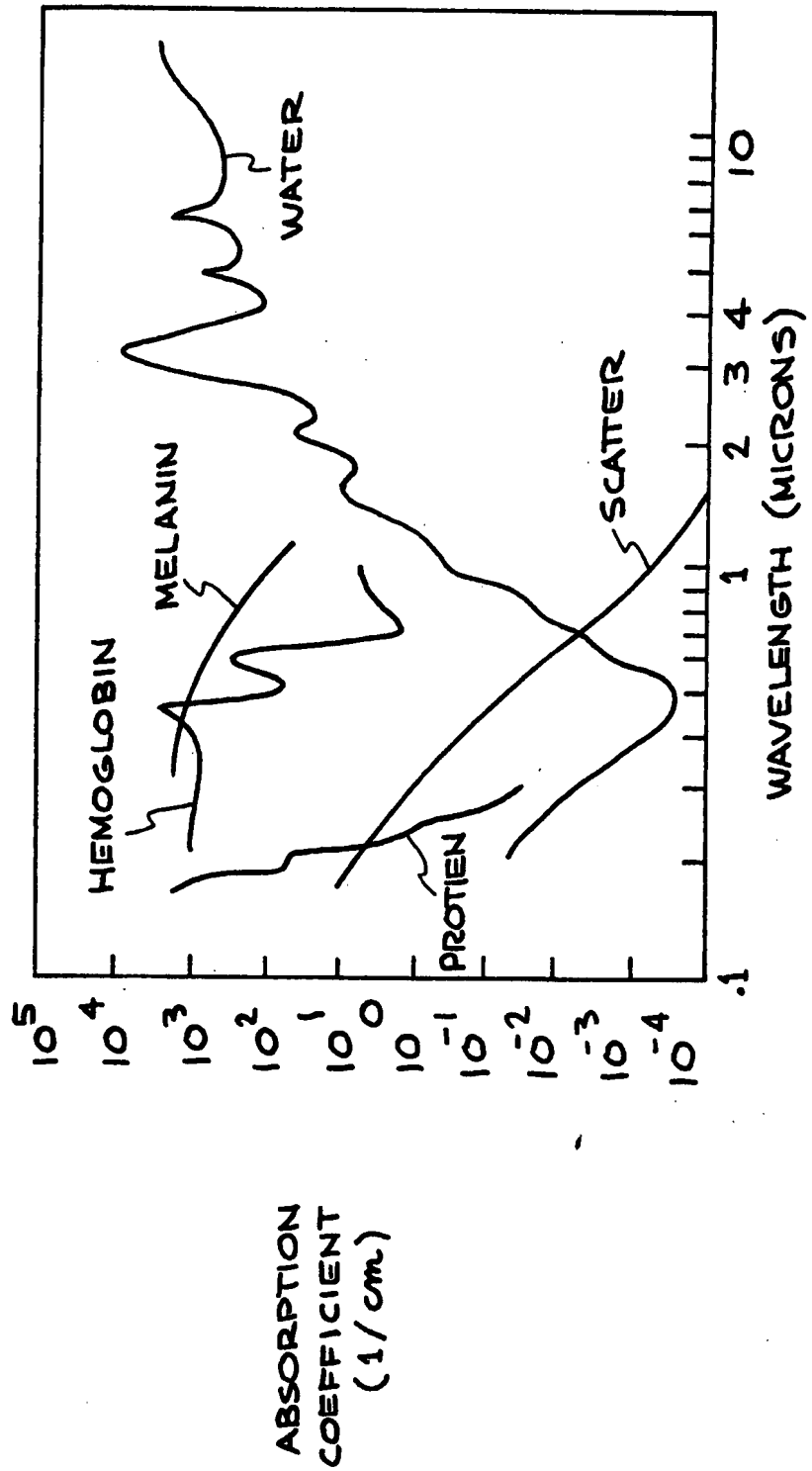

HEMOGLOBIN
MELANIN
WATER
SCATTER
PROTIEN
WAVELENGTH (MICRONS)
.1
1
2
3
4
10
ABSORPTION COEFFICIENT (1/cm)
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}

FIG. 3

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/IB96/00631

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12M 1/02
US CL :435/287.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,498,392 A (WILDING ET AL.) 12 March 1996 (12.03.96), see entire document.	1-21
Y	US 5,252,294 A (KROY ET AL.) 12 October 1993 (12.10.93), see entire document.	1-21
Y	US 4,821,997 A (ZDEBLICK) 18 April 1989 (18.04.89), see entire document.	1-21
Y	US 4,927,766 A (AUERBACH ET AL.) 22 May 1990 (22.05.90), see entire document.	1-21
A	US 5,292,362 A (BASS ET AL.) 08 March 1994 (08.03.94), see entire document.	1-21
A	US 5,367,878 A (MUNTZ ET AL.) 29 November 1994 (29.11.94), see entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 OCTOBER 1996	Date of mailing of the international search report 31 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM H. BEISNER Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB96/00631

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STIX. Gene Readers: Microelectronics has begun to merge with biotechnology. Scientific American. January 1994, pages 149-150, see entire document.	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB96/00631

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/286.1, 287.1, 287.2, 288.3, 288.4, 288.5, 289.1, 292.1, 305.1-305.4, 808; 422/58, 68.1, 82.05, 102; 219/201; 250/429, 432R, 461.1, 461.2